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Growth Dynamics of Domains in Ternary Fluid Vesicles

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飽和リン脂質、不飽和リン脂質、コレステロールからなる３成分ベシクルにおける液体ドメインの成長ダイナミクスを蛍光顕微鏡観察した結果、*Normal coarsening*と*Trapped coarsening*に分けられた。Normal coarseningを示すベシクルは余剰面積をほぼ持たず、ドメインは２次元のスケーリング則 $t\sim D^2 lnD$ (Dはドメインサイズ) に従ってBuddingせずに成長する。しかし成長の初期過程では流体力学的相互作用によるずれが生じる。一方、Trapped coarseningを示すベシクルは大きな余剰面積を持ち、ドメインはあるサイズまで成長するとBuddingしTrapされる。Buddingしたドメインが近づくと、ドメイン間に挟まれたマトリックスの曲げエネルギーが増大し、ドメインの合体を妨げる斥力を生む。Buddingしたドメインを持つベシクルの自由エネルギーの解析から、ベシクルの持つ余剰面積がある値よりも大きい時、余剰面積が大きい程コースニングのより初期課程でBuddingしTrapされることが分かり、実験結果を説明することが出来た。

Introduction

Over the past few years, the liquid domain formation has been observed in giant vesicles consisting of saturated phospholipid, unsaturated phospholipid, and cholesterol using a fluorescence microscopy [1, 2]. When the temperature is dropped from the homogeneous one phase region to the two phase region, the membrane is separated into a saturated lipid and cholesterol rich phase and an unsaturated lipid rich phase. During the phase separating process, the circular domains develop into a small number of large domains by colliding and coalescing with other domains. One of the interesting features of the phase separation on a vesicle is the coupling of the phase separation and the membrane elasticity. With the growth of domains, the interfacial energy of domains starts to play a dominant role on the domain morphology, which makes the domains bud towards the outside of the vesicle [3]. In this paper we present a systematic experimental investigation on the growth dynamics of the domains in the ternary fluid vesicles using the fluorescence microscopy.

Experiment

Giant vesicles consisting of saturated phospholipid (DPPC), unsaturated phospholipid (DOPC), and cholesterol (4 / 4 / 2) were prepared by a gentle hydration method. To observe the both domains and matrix phases with the fluorescence microscopy, we added Texas Red DHPE (TR-DHPE) and Perylene as a dye. TR-DPPE is localized in a DOPC-rich phase and shows a red color, whereas Perylene partitions preferentially into a DPPC-and-cholesterol-rich phase and shows a blue color. For the observation of the phase separating vesicles, we decreased the temperature from the one phase region to the miscibility transition temperature. When the phase separation took place, we fixed the temperature immediately and the time evolution of domains was recorded with a CCD camera (Carl Zeiss, Axio Cam). In order to estimate the area-to-volume ratio of a vesicle, we adopted a micro-pipette aspiration technique. By the micro-pipette aspiration, a flaccid spherical vesicle deforms to a tight vesicle. Then we are able to calculate the total volume and total surface area of the vesicle.

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Result and Discussion

We have investigated the growth dynamics of circular domains in the ternary vesicle consisting of DPPC, DOPC and cholesterol. The coarsening process can be categorized into two types; the *normal coarsening* and the *trapped coarsening*. In order to make clear the difference between the normal coarsening and the trapped coarsening, we plot the time evolution of the mean domain diameter $D(t)$ in Fig. 1 and show the fluorescence images for the both cases in Fig. 2. For the normal coarsening, mean domain diameter $D(t)$ grows following the two-dimensional scaling law; $t \sim D^2 / \ln D$ in a diffusion-and-coalescence manner. However at the early coarsening stage, the experimental growth law deviates from the theoretical prediction due to the hydrodynamic interactions. For the trapped coarsening, the domains grow to a certain size, and then the coarsening is suppressed for a long time. As shown in the two-color images of Fig. 2, the apparent difference between the two types is the budding. If the domains bud, the bending of the matrix membrane between the two approaching budding domains induces the repulsive inter-domain interaction, which brings the domains trapping. The key condition for the budding is the amount of the excess area. In fact, the excess area of the vesicle is small for the normal coarsening, and large for the trapped coarsening. The observed phenomenon is examined in terms of the free energy for a vesicle with budding domains under the area-to-volume constraint. The theoretical calculation based on the budding model reveals that the condition determining whether the vesicle shows the trapped coarsening or the normal coarsening depends on the excess area of the vesicle, the line tension between phase separating domains and the bending modulus of the membrane [4].

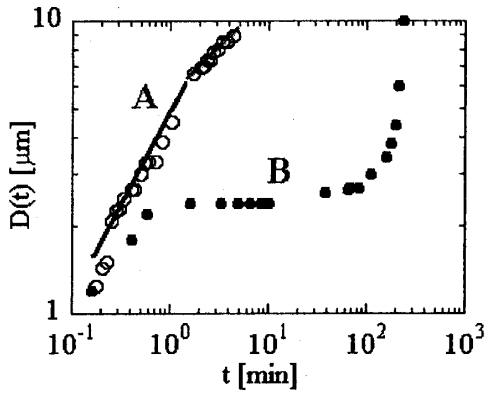

Fig. 1 The time evolution of the domain growth on a vesicle, (A) the normal coarsening and (B) the trapped coarsening.

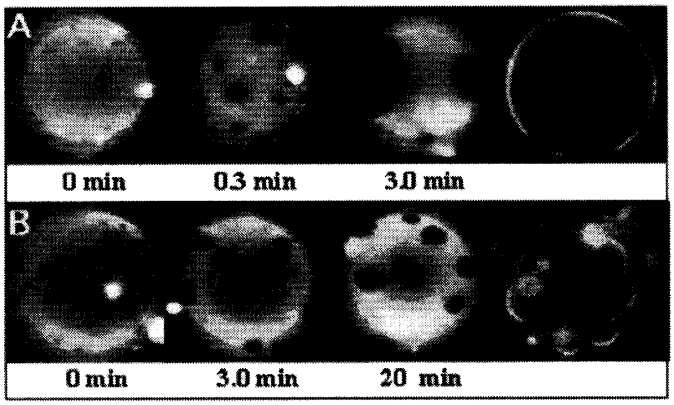

Fig. 2 The time evolution of the fluorescence images for (A) the normal coarsening and (B) the trapped coarsening.

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